

In the Specification:

Please amend the specification as follows.

Please replace the paragraph entitled, “Nucleic Acid Isolation and Amplification,” bridging pages 11 and 12 with the following new paragraph:

Nucleic Acid Isolation and Amplification

Total RNA and genomic DNA from: normal human thyroid, thyroid tumors, and cell lines (treated with agents described above), were isolated by the acid-guanidinium-phenol-chloroform method (21). All surgical samples were snap-frozen and stored at -80°C until processed by homogenization in Trizol reagent (Life Technologies, Gaithersburg, MD) while still frozen. Complementary DNA (cDNA) was synthesized from 1.0 µg of total RNA using MMLV reverse transcriptase (RT) with random-hexamer primers (Clontech, Palo Alto, CA). Each 50 µL polymerase chain reaction (PCR) vessel contained: 60 mM Tris HCl, pH 9.0, 15 mM ammonium sulfate, 3.5 mM MgCl₂ (1.5 mM for hTTF-1), 250 µM dNTPs (Boehringer-Mannheim, Indianapolis, IN), 0.2 µM each primer pair, 1 U AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, CT), 0.2 µg TaqStart Antibody (Clontech) and 3% cDNA. β-actin amplification (primers, Stratagene, La Jolla, CA) confirmed cDNA integrity, purity, and template equivalence for semiquantification. PCR primers (upstream 5' to 3' / downstream 5' to 3', in all cases) used for amplification were, for hNIS (5), CTGCCCCAGACCAGTACAT GCC (SEQ ID NO. 1)/ TGACGGTGAAGGAGCCCTGAAG (SEQ ID NO. 2) (to amplify a coding region spanning four introns (4) yielding a 303 bp product from cDNA) and for Pax-8, AAGTCCAGCATTGCGGCACA (SEQ ID NO. 3)/ GAGGGAAGTGCTTATGGTCC (SEQ ID NO. 4)(22) to amplify a 329 bp product). Amplification conditions for hNIS and Pax-8 were: denaturation (95°C x 5 min); 40 cycles of 20 sec at 95°C and 60 sec at 68° C; followed by extension at 72°C for 3 min. The hTTF-1 product was amplified with intron spanning primers, GCCGTACCAGGACACCATGAG (SEQ ID NO. 5)/ CAGGTACTTCTGTTGCTTGAAG (SEQ ID NO. 6), which amplify a 263 bp fragment. The conditions were: 95°C for 5 min; 45 cycles of 95°C for 20 sec, 60°C for 60 sec, and 72°C for 30 sec; followed by extension at 72°C for 3 min. The RT-PCR products were resolved on 2% agarose gels and visualized by ethidium bromide staining.

Replace the paragraph entitled “Methylation -Specific Polymerase Chain reaction (MS-PCR Analysis)” bridging pages 12 and 13 with the following new paragraph:

Methylation-Specific Polymerase Chain Reaction (MS-PCR) Analysis

This method utilizes PCR primer pairs to distinguish methylated from unmethylated DNA in bisulfite-modified target DNA, in which bisulfite converts unmethylated cytosines to uracil (23,24). Genomic DNAs, from normal and tumoral human thyroid tissues and cell lines, were isolated by standard techniques (21) and 1.0 µg aliquots were denatured by NaOH (10 min at 37°C), then treated with 10 mM hydroquinone and 3.0 M sodium bisulfite (pH 5.0 under mineral oil for 16 hrs at 50°C). Modified DNA was purified on a resin column (Qiagen) and further treated with 0.3 N NaOH for 5 min prior to ethanol precipitation. The PCR mixture contained 16.6 mM ammonium sulfate, 67 mM Tris-HCl (pH 8.8), 6.7 mM MgCl₂, 10 mM β-mercaptoethanol, 1.25 mM dNTPs, 0.2 µL TaqStart antibody, 1 U AmpliTaq DNA polymerase, 10 pmoles each of sense and antisense methylation-specific primers, and 50 mg of bisulphite-modified DNA target. Primers used for analysis of the hNIS promoter CpG island methylation were selected for cytosine-rich regions, containing CpG dinucleotides near the 3' end of the primers, hNIS-MET-P (sense, 5' to 3', TTAGGTTTGGAGGCGGA GTCGC (SEQ ID NO. 7) and antisense, 5' to 3', ACCGACTATCTATCCCT CTCCCTAAACG) (SEQ ID NO. 8) for a 143 bp product from methylated DNA and hNIS-UNMET-P (sense, 5' to 3', TTGTTTTTAGGTTTGGAGGTG GAGTTGT (SEQ ID NO. 9) and antisense, 5' to 3', CAACCAACTATCTATCCCTCTC CCTAAACA) (SEQ ID NO. 10) for a 151 bp product from unmethylated genomic DNA. Additional sets of primers were similarly designed to analyze further downstream elements. They were: hNIS-MET-L (sense, ATAGATAGATAGTAGGGGCGGAC (SEQ ID NO. 11) and antisense, GACCT CCATAAAAACGAATACG) (SEQ ID NO. 12) for a 265 bp product, with hNIS-UNMET-L (sense, TAGGATAGATAGATAGTAGGGGTGGAT (SEQ ID NO. 13), and antisense, CTCCACAACCTCCATAAAAACAAATACA) (SEQ ID NO. 14), for a 275 bp product, hNIS-MET-C (sense, AGGTCGTGGAGATCGGGGAAC (SEQ ID NO. 15) and antisense, ACGATAAACCTCCGACGACACG) (SEQ ID NO. 16) for a 242 bp product, and hNIS-UNMET-C (sense, TTATGGAGGTTGTGGAGATTGGGGAAT (SEQ ID NO. 17), and antisense, CATAACAATAAACCTCCAACAACACA) (SEQ ID NO. 18), for a 252 bp product. The amplification conditions were: Taq polymerase activation at 95°C for 5 min, 40 cycles of: denaturation at 94°C for 20 s, annealing at 60°C for 30 s and polymerization at 72°C for 30 s. MS-PCR products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining and UV transillumination.